Type 4 Phosphodiesterase Inhibitors Have Clinical and *In Vitro* Anti-inflammatory Effects in Atopic Dermatitis

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Increased cyclic AMP-phosphodiesterase activity in peripheral blood leukocytes is associated with the immune and inflammatory hyperreactivity that characterizes atopic dermatitis. Atopic phosphodiesterase has high sensitivity to a variety of enzyme inhibitors, suggesting an increased therapeutic advantage. The objective of this study was to use *in vitro* assays to identify a potent phosphodiesterase inhibitor and then to investigate its effectiveness in treating atopic dermatitis.

Leukocyte enzyme activity was measured by radioenzyme assay, whereas prostaglandin E_2 and interleukins 10 (IL-10) and 4 (IL-4) were measured in 24-h culture supernatants of mononuclear leukocytes by immunoassays. The effect of a topical phosphodiesterase inhibitor on atopic dermatitis lesional skin was assessed by double-blind, paired comparisons of active drug and placebo ointments applied to symmetrically involved sites over a 28-d period.

Using *in vitro* assays, we demonstrated the ability of selective high-potency phosphodiesterase inhibitors to reduce prostaglandin E_2, IL-10, and IL-4 production in atopic mononuclear leukocyte cultures. We selected the Type 4 phosphodiesterase inhibitor, CP80,633, based on its inhibitory potency, for clinical testing by topical, bilateral paired comparisons in 20 patients with atopic dermatitis and demonstrated significant reductions of all inflammatory parameters.

Phosphodiesterase inhibitors modulate several pathways contributing to the exaggerated immune and inflammatory responses, which characterize atopic dermatitis. This *in vivo* demonstration of anti-inflammatory efficacy may provide a useful alternative to the over-reliance on corticosteroid therapy in atopic disease. *Key words: PDE/IL-4/IL-10/monocytes. J Invest Dermatol 107:51–56, 1996*

Cyclic nucleotide phosphodiesterases comprise a family of isoenzymes that hydrolyze the 3′,5′-cyclic nucleotides to 5′-nucleotide monophosphates (Beavo, 1988). We have been particularly interested in cAMP phosphodiesterase (PDE), because of the increased cAMP hydrolytic activity in leukocytes from patients with atopic dermatitis (AD), asthma, and allergic rhinitis (Grewe *et al*, 1982). These diseases represent a symptom complex characterized by immunologic hyper-reactivity and by inappropriate inflammatory cell infiltration into skin and respiratory tissues. The abnormal PDE activity correlates with leukocyte functional defects including basophil histamine hyper-releasibility (Butler *et al*, 1983) and increased B-lymphocyte IgE production (Cooper *et al*, 1985), both of which are normalized by *in vitro* incubation with the PDE inhibitor (PDE-i), Ro 20-1724.

Phosphodiesterases have in recent years been classified into seven families (Types I–VII or, by genome terminology, PDE1–7) according to a number of characteristics including sensitivity to inhibitors (Beavo, 1990). In previous studies, we found evidence that the more active PDE4 in atopic leukocytes had increased sensitivity to inhibition by Ro 20-1724 and other agents (Giustina *et al*, 1984; Chan and Hanifin, 1993), compared to PDE in normal leukocytes. We have utilized this technique to assay the potency of PDE4 inhibitors, comparing effects on PDE activity in atopic and normal mononuclear leukocytes (MNL) (Chan and Hanifin, 1993). In this study, we conducted an *in vitro* survey of several compounds shown to be potent PDE4 inhibitors. Among these, we found that two enantiomers, CP80,633 (Cohan *et al*, 1995) and CP102,995, and the racemate, CP76,593, had the highest potency in comparison to other agents. Consistent with past studies (Giustina *et al*, 1984; Chan and Hanifin, 1993), these compounds showed a greater relative specificity for the atopic compared to the normal PDE isoenzyme. These techniques appear to provide a relevant *in vitro* system for predicting the therapeutic efficacy of each new PDE-i in the management of AD, asthma, and other inflammatory diseases. Focusing on the higher potency inhibitors of PDE, we assayed the effectiveness of new compounds on eicosanoid and cytokine production *in vitro*. We then carried out a double-blind, vehicle-controlled, paired-comparison study to assess the safety and efficacy

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Abbreviations: PDE, phosphodiesterase; AD, atopic dermatitis; PDE-i, phosphodiesterase inhibitor; MNL, mononuclear leukocytes; FBS, fetal bovine serum; IFN-γ, interferon-γ; Th1, Type 1 T helper cells; Th2, Type 2 T helper cells; ELISA, enzyme-linked immunosorbent assay.

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of one of the new compounds when applied topically for treatment of AD. Our studies demonstrate that these PDE inhibitors modulate multiple immune and inflammatory pathways and significantly reduce signs and symptoms of atopic inflammation.

MATERIALS AND METHODS

RPMI-1640 medium, Gey's balanced salt solution, fetal bovine serum (FBS), neuraminidase, Hanks' balanced salt solution, Hanks' calcium/magnesium-free balanced salt solution: Gibco (Grand Island, NY). 5'-Nucleotidase, cAMP, imidazole, and snake venom (5'-nucleotidase): Sigma Chemical Co., St. Louis, MO. Hypaque-Ficoll: Pharmacia, Piscataway, NJ. [^{3}H]cAMP (36 Ci/mmol): New England Nuclear, Boston, MA. Ion exchange resin AGIX2 (200–400 mesh): Bio-Rad, Richmond, CA. "Ready-Solv" scintillation fluid: Beckman, San Jose, CA. Anti-CD3 (OKT3): Ortho Diagnostics, Raitway, NJ. Ro 20-1724 was a gift from Hoffmann LaRoche, Nutley, NJ. CP76,593 and its resolved enantiomers, CP80,633 (Cohan *et al*, 1995) and CP162,995, were received from Central Research Division, Pfizer Inc., Groton, CT.

Subjects All subjects gave informed consent approved by the institutional Human Research Committee. For leukocyte studies, venous blood was drawn at 8:00 a.m., and immediately mixed with heparin (10 units/ml) for further processing. Normal, healthy subjects had no personal history of asthma, allergic rhinitis, or AD. Patients with active AD were chosen according to well-defined criteria (Hanifin and Rajka, 1980) and blood donors had moderate to severe disease. Blood donors' ages ranged from 25 to 52 years (9 males and 11 females) for normal subjects and 20 to 50 years for AD subjects (10 males and 8 females). Individual experiments were not precisely age- and gender-matched, because leukocytes were used in various assays on any given day. Subjects for the clinical trial had lesions not exceeding 20% of total body surface area. No donors had received antihistamines or topical corticosteroid therapy for at least 96 h prior to study, and none had used systemic adrenergic, PDE inhibitors, or corticosteroid medications for at least 1 mo. No caffeine or other methylxanthine-containing beverages were consumed within 14 h prior to leukocyte studies.

Cell Preparations Blood was separated by Hypaque-Ficoll gradient centrifugation at 400 × *g* for 30 min, and MNL were harvested from the interphase of plasma and separation fluid (Chan and Hanifin, 1993). The cells were washed three times with saline and spun at 400, 300, and 250 × *g* sequentially to eliminate platelet contamination. MNL were harvested and counted using a Coulter counter. Differential-lymphocyte and -monocyte quantitations utilized Giemsa and acid naphthyl acetate esterase stains and latex bead phagocytic ingestion. These quantitations were monitored in all preparations and showed no differences between AD and normal subjects in terms of percentages of monocytes and lymphocytes. MNL were either freeze-thawed three times in an acetone-dry ice bath, and the homogenates were stored at −80°C until assayed for homogenate PDE activity, or cultured as described below. To obtain monocytes, MNL at 4 × 10^6 cells/ml were allowed to adhere in a 16- × 100-mm petri dish for at least 2 h at 37°C in RPMI-1640 + 10% FBS. The nonadherent cells were decanted and washed three times with warm Gey's balanced salt solution. The adherent monocytes were recovered by scraping with a sterile rubber policeman. The MNL compositions, determined by Stat Stain (Volu-Sol; Logos Scientific, Henderson, NV), contained 10–40% monocytes, 60–70% lymphocytes, ≤2% polymorphonuclear leukocytes, and ≤5% platelets. The nonadherent cells were typically ≥95% $CD3^+$ lymphocytes. Cell viability, monitored by trypan blue exclusion test, was always >98%. Monocyte purity in the adherent cell preparations, confirmed by acid naphthyl acetate esterase and Factor XIII immunoperoxidase staining, was ≥95%.

For PGE_2 production, monocytes (2 × 10^6/ml) were incubated in RPMI-1640/10% FBS. For IL-4 production, MNL (2 × 10^6/ml) were incubated in RPMI-1640/10% FBS with 10 ng/ml anti-CD3 (Chan *et al*, 1993). After 24 h, supernatants were harvested by pelleting cells at 700 × *g*.

PDE Inhibition/Assay Homogenized leukocyte preparations were kept at 4°C, and various PDE inhibitors were immediately added. All inhibitors were dissolved in 50% ethanol at a concentration of 10^{-2} M, then further diluted in Gey's balanced salt solution to appropriate concentrations. For the determination of IC_{50}, 10^{-8} to 10^{-3} M final concentrations of inhibitors or control buffer were used. The mixtures were then incubated at 37°C for 60 min. PDE activities were determined in the presence of the inhibitors or in control buffers in all experiments. Ro 20-1724 was used as a reference compound for comparison of inhibitor effects in all studies. Maximum inhibition for each compound was determined by curve-fitting with a computer program, and the concentration giving 50% of maximum inhibition was recorded as IC_{50}.

PDE was assayed using a modified method of Thompson *et al* (1979). The incubation mixture (0.4 ml) contained 1 μM cAMP, 200,000 cpm of [^{3}H]cAMP, and 0.2 ml of sample (10^7 cells/ml) or standard PDE in 40 mM Tris-Cl buffer (pH 8.0) containing 3.75 mM β-mercaptoethanol and 15 mM $MgCl_2$. After incubation at 30°C for 10 min, the reaction was terminated by snap-freezing in ethanol-dry ice bath and the mixture was then boiled for 1 min. Purified 5'-nucleotidase was added to the mixtures, which were then further incubated for 10 min at 30°C and then transferred to Pasteur pipette columns containing ion exchange resin AGIX2 to remove the remaining nucleotides and nucleosides. The radioactivity in the eluates was quantitated in scintillation fluid. Enzyme activity was expressed as picomoles of cAMP hydrolyzed per μg of protein. Protein concentration was determined by an assay using Bio-Rad protein dye. A standard PDE from bovine heart was used to monitor consistency and recovery in each assay (Chan and Hanifin, 1993).

Immunoassays Prostaglandin E_2 (PGE_2) was assayed by radioimmunoassay as previously described (Chan *et al*, 1993), using culture supernatants containing 0.05 mM indomethacin as blank. PGE_2 antisera were produced in rabbits according to the method of Jaffe and Behrman (1974). PGE_2 was conjugated to porcine thyroglobulin by the mixed anhydride method before immunization of the rabbits. At a dilution of 1:6000, the PGE_2 antiserum had a sensitivity of 10 pg/0.1 ml of sample and the following cross-reactivities at B/B_0 50% were: PGD_2, 0.8%; $PGF_{2\alpha}$, 1%; $PGF_{1\alpha}$, 0.3%; PGE_1, 9.3%; 6-keto-$PGF_{1\alpha}$, 2.2% and 6-keto-PGE_2, 2%, as previously reported (Geissler *et al*, 1989).

Quantitative determination of human IL-4 was performed using enzyme-linked immunosorbent assay (ELISA) kits (R & D Systems, Minneapolis, MN) with modification to increase sensitivity (Li *et al*, 1993). The practical sensitivity of the assay was 5 pg/ml. In order to measure below 5 pg/ml, the ELISA procedure was modified, according to manufacturer's instructions, by extending the color development time at low concentrations from 15 to 45 min to generate a linear scale between 0 and 5 pg/ml. A human IL-10 ELISA kit was also used to determine IL-10 concentrations. The lower limit of detection was 7.8 pg/ml for undiluted monocyte culture supernatants. PDE inhibitors did not interfere with the IL-10 standard curves.

Topical Therapy Twenty patients with AD (12 males and 8 females) aged 18 to 50 years were enrolled in the study to determine efficacy of topical CP80,633 ointment for treatment of skin inflammation. Exclusions included females with childbearing potential, pregnant or nursing women, and patients who required any medication that might interact with or obscure treatment effects including the use of oral theophylline derivatives, oral, parenteral, or topically applied corticosteroids, and H1 or H2 antihistamines. Patients had to be in good health with normal laboratory parameters and electrocardiograms.

The study design was a right/left paired-comparison study to compare the efficacy of topically applied CP80,633 (0.5%) ointment twice daily for 28 d with its petrolatum vehicle on 200-cm^2 lesional areas. Active drug and vehicle were assigned by side in a randomized, double-blind fashion. Patients were selected for the presence of symmetrically involved anatomical sites on the right and left sides excluding the hands, feet, and face. Grading each of three inflammatory parameters [(i) erythema; (ii) induration/papulation; (iii) excoriation] utilized a scale from 0 to 3 (1 = mild, 2 = moderate, 3 = severe, with half steps) and baseline scores were required to be at least 6 of the possible 9 for the total clinical score. The subjective itch score was likewise graded on a scale of 0–3. After physical examination, blood chemistries [alkaline phosphatase, alanine and aspartate aminotransferase (ALT and AST), Na^+, K^+, Cl^-, glucose, uric acid, blood urea nitrogen, creatinine, and total bilirubin], urinalysis and hematology (complete blood count with differential) were obtained from venous blood. Study drug was applied twice daily for 28 d of treatment. Overall efficacy assessments were made at days 3, 7, 14, 21, and 28 to grade improvement or worsening of inflammatory signs, determined by comparing to baseline scores the specific parameters and the total clinical score. Repeat laboratory evaluations were performed at days 7, 14, and 28 or at the time of early discontinuation. Electrocardiogram was repeated at the end of study.

Statistical Analysis For *in vitro* studies comparing IC_{50} values of PDE and IL-4 production in cultures, Student's *t* test was used, whereas Mann-Whitney nonparametric analysis was used in comparing effects of PDE inhibitors on PGE_2 and IL-10 production. For clinical studies, individual parameter scores and the sum of scores were compared for active and placebo-treated sites. The score at each time point was subtracted from the baseline score and this change was analyzed using two-sided *t* tests,

Table 1. Comparison of 50% Inhibition Concentrations (IC_{50}) Against Phosphodiesterase Activity in Homogenates of Mononuclear Leukocytes from Patients with Atopic Dermatitis (AD) and from Normal Subjects*

Phosphodiesterase Inhibitors	IC_{50} (μM, mean ± SEM) AD (n)†	Normal (n)	Values‡
Pentoxyfylline	1.76 ± 0.26 (4)	3.58 ± 1.22 (3)	0.048
Rolipram	0.86 ± 0.22 (7)	1.13 ± 1.86 (7)	0.037
Theophylline	27.11 ± 12.43 (7)	87.92 ± 19.68 (9)	0.027
Ro 20-1724	0.17 ± 0.08 (8)	2.9 ± 0.55 (8)	0.0076
CP76,593	0.32 ± 0.12 (11)	4.43 ± 1.60 (5)	0.00176
CP80,633	0.015 ± 0.003 (10)	ND	
CP102,995	0.88 ± 0.607 (8)	ND	

* Ficoll-Hypaque gradient-separated peripheral blood mononuclear leukocytes were homogenized and incubated with final concentrations of each inhibitor ranged from 10^{-9} to 10^{-4} M to determine their IC_{50} for the inhibition of PDE activity.

† (n) = no. of donors; ND = not done.

‡ p values were determined by Student's t test comparing IC_{50} between normal and AD.

employing a level of significance ($p < 0.05$) to test the alternative hypothesis that the mean paired difference is not equal to zero.

RESULTS

Greater Type 4 Inhibition of Atopic PDE Our first objective was to compare potencies of newer Type 4 inhibitors with presently available agents on PDE activity in MNL homogenates. We initially determined mean IC_{50} values of PDE inhibition in MNL homogenates by the racemic mixtures CP76,593, for both normal and AD groups **(Table I)**, and compared these mean IC_{50} values from AD and normal groups with those of pentoxyfylline, rolipram, theophylline, and Ro 20-1724. Results showed that CP76,593, which was slightly less potent than Ro 20-1724, was more active against the AD isozyme than the other inhibitors. Each of these compounds had less potency against the normal isozyme, consistent with our previous findings (Giustina *et al*, 1984; Chan and Hanifin, 1993).

We then compared pure enantiomers (+) CP80,633 and (−) CP102,995, with (±) CP76,593 and found that CP80,633 was 21- and 59-fold more inhibitory than either CP76,593 ($p < 0.01$) or CP102,995 ($p = 0.008$), respectively, against the PDE in MNL from patients with AD **(Table I)**. CP80,633 was also significantly more active than Ro 20-1724 ($p < 0.001$). Because the focus of our studies was AD inflammation, pure enantiomers were not tested on normal cells.

PDE Inhibitors Reduce Atopic Monocyte PGE_2 and IL-10 Production We recently reported increased spontaneous PGE_2 (Chan *et al*, 1993) and IL-10 (Ohmen *et al*, 1995) production by cultured monocytes from patients with AD. We found that these increases corresponded with elevated PDE activity and hypothesize that this results in inadequate cAMP modulation of monocyte function (Chan *et al*, 1993). To evaluate the effect of PDE inhibitors, PGE_2 levels were measured by radioimmunoassay in 24-h culture supernatants from monocytes treated with CP80,633 (0.1 μM) or Ro 20-1724 (1 μM), comparing AD and normal preparations. The concentrations of the inhibitors used were chosen for their respective maximal effects, as determined by dose-response curves. Untreated control cultures confirmed previous findings of markedly elevated mean PGE_2 levels in AD preparations (562 ± 107 *versus* 89 ± 27 for normals, mean ± SEM $p = 0.0036$; **Fig 1**). Both CP80,633 and Ro 20-1724 caused significant reductions in PGE_2 supernatant levels in AD compared to the untreated control culture ($n = 4–8$). Ro 20-1724 did not affect PGE_2 production in normal cell cultures. This was consistent with the reduced enzyme inhibition in normal cells **(Table I)** and with previous findings of increased inhibitor sensitivity of atopic PDE (Giustina *et al*, 1984; Chan and Hanifin, 1993). The PGE_2 changes

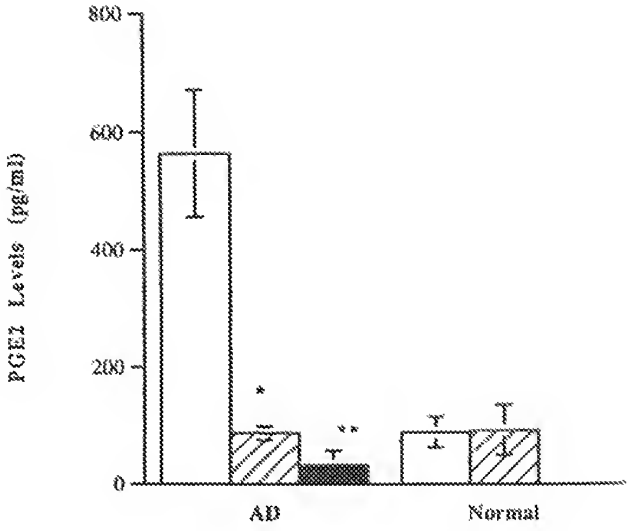

Figure 1. Prostaglandin E_2 levels in monocyte culture supernatants. Prostaglandin E_2 (PGE_2) levels (pg/ml, mean ± SEM) in unstimulated culture supernatants from atopic dermatitis (AD) and normal monocytes cultured for 24 h with media alone (□), Ro 20-1724 (1 μM, ▨), or CP80,633 (0.1 μM, ■; not done in normals). *p = 0.004, †p < 0.001.

in Ro 20-1724 and CP80,633-treated AD cell cultures were significant ($p < 0.001$ and < 0.001, respectively) by Mann-Whitney.

We also compared Ro 20-1724 and CP80,633 inhibition of AD monocyte IL-10 production in a dose-response experiment **(Fig 2)**. The concentration of CP80,633 required for 50% inhibition of IL-10 (IC_{50}) was 2.2 nM, indicating a 1000-fold greater potency than Ro 20-1724 ($IC_{50} = 2.5$ μM) in reducing production of this cytokine ($p < 0.001$, by Mann-Whitney test). Initial experiments using the usual 10^{-7} M CP80,633 concentration showed 94% and 108% inhibition of spontaneous IL-10 production in normal ($n = 2$) and AD ($n = 4$) monocyte cultures, respectively. Mean spontaneous IL-10 production in these studies by normal monocytes was 578 ± 118 pg/ml ($n = 2$) and 1962 ± 276 pg/ml ($n = 5$) by AD monocytes.

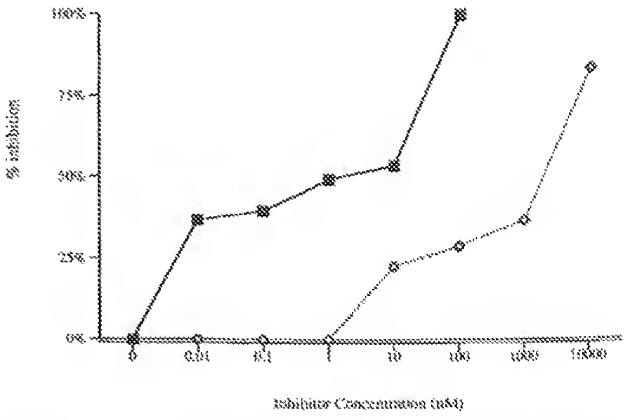

Figure 2. Dose effect of PDE inhibitors on monocyte IL-10 production. Dose-related inhibition of spontaneous atopic monocyte IL-10 production by Ro 20-1724 (○) and CP80,633 (■). Spontaneous IL-10 production for this donor was 2330 pg/ml.

Table II. PDE Inhibitor Effects on IL-4 Production[a]

	AD (n)	p value[b]	Normal (n)	p value[b]
Control	42.6 ± 6.7 (8)		17.6 ± 1.9 (6)	
+ Ro 20-1724	5.2 ± 2.1 (6)	0.003	14.2 ± 1.6 (6)	NS[c]
+ CP80,633	2.4 ± 2.5 (3)	0.036	21.9 ± 4.6 (3)	NS

[a] Mononuclear leukocytes were cultured for 24 h with anti-CD3 (10 ng/ml) ± Ro 20-1724 (1 μM) or CP80,633 (0.1 μM). Supernatant IL-4 levels were measured by ELISA and expressed as pg/ml mean ± SEM.

[b] p value comparing inhibitor effect to controls.

[c] NS = not significant.

Reduced IL-4 Production We previously noted an inverse relationship between elevated PDE activity and reduced interferon-γ (IFN-γ) levels in atopic MNL (Chan *et al*, 1993) and reasoned that increased IL-4 production might likewise relate to abnormal PDE activity. We assessed the effect of PDE inhibitors on IL-4 production determined by ELISA from supernatants of anti-CD3-stimulated AD and normal 24-h MNL cultures, with and without PDE inhibitors. As can be seen in **Table II**, both Ro 20-1724 (1 μM) and CP80,633 (0.1 μM) caused 8- and 18-fold reductions, respectively, in AD supernatant concentrations. These inhibitors had no effect on normal IL-4 supernatant levels.

Topical CP80,633 as an Anti-Inflammatory Agent Based on its demonstrated PDE inhibitory potency, CP80,633 was selected for clinical testing. Twenty patients with AD were enrolled in a clinical trial to assess the efficacy and safety of CP80,633. Symmetrical, moderately involved areas of up to 200 cm^2 on each of the right and left sides were selected for assessment of active *versus* placebo therapy. The baseline mean total scores at bilateral sites were comparable (5.20 ± 0.22 *versus* 5.33 ± 0.19, $p = 0.27$). Efficacy evaluations were recorded at days 3, 7, 14, 21, and 28 during therapy. Inflammation was quantitated by the same observer grading erythema, induration/papulation, and excoriation on a scale from 0 to 3 (none, mild, moderate, or severe). Baseline scores compared with those at day 28 showed significant improvement on sites receiving CP80,633 with mean reductions in erythema ($p = 0.004$), induration ($p < 0.001$), and excoriations ($p = 0.046$), the latter serving as an objective indicator of pruritus. Patients were also asked to estimate the level of itching at each visit, and their subjective responses likewise showed significant improvement on active compared to placebo-treated sites ($p = 0.002$). The response to active drug was consistent among the subjects, with an improvement in total clinical score observed in 16 of 20 CP80,633-treated sites (**Fig 3**), graded at the last assessment, compared to only three of 20 placebo-treated sites (mean ± SD 1.40 ± 1.76 and −0.65 ± 1.48, respectively; $p < 0.001$).

Figure 4 shows the mean change from baseline of the total clinical scores (erythema + induration + excoriation) of active and placebo-treated sites over the course of the study. Mean baseline scores were similar for vehicle and CP80,633-treated sites. Significantly reduced inflammation was evident as early as day 3 and continued throughout the therapy phase for actively treated sites, which demonstrated significantly greater improvement than placebo sites at each time point. Because we had previously detected *in vitro* evidence of tachyphylaxis among asthmatic patients treated with theophylline long-term (Giustina *et al*, 1984) we were interested in whether this might occur with topical CP80,633 therapy; however, improvement continued throughout the 4-wk course of treatment with active drug.

Adverse events indistinguishable from manifestations of AD (itching, burning, folliculitis) were noted as single events on nine placebo- and nine vehicle-treated sites, and 16 of these 18 observations were bilateral. In two instances, folliculitis was noted on the active drug-treated site and in two other patients, bilateral folliculitis was noted. These events are typical of AD (Hanifin and Rajka, 1980) and the occurrence of folliculitis in 5.3% of active site observations was not significantly greater than the 2.6% frequency on vehicle-treated sites. There were no drop-outs for adverse events and no clinically relevant laboratory or electrocardiogram changes. Eight patients discontinued before the end of the treatment period, all due to flaring of dermatitis on untreated areas (no other therapy was allowed during the course of the trial). In three of the eight patients, dermatitis became intolerable on one side, the placebo-treated side; in the other five patients, the study was

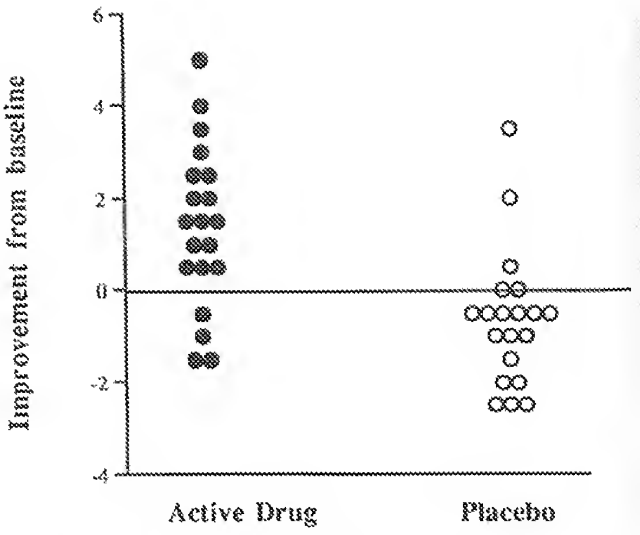

Figure 3. Clinical effect of topical PDE inhibitor, CP80,633. Clinical effect (change in mean total score of clinical parameters) of topical phosphodiesterase inhibitor, CP80,633, on bilateral atopic dermatitis lesions. Each point represents the difference between total clinical score at baseline and the score at the last observed assessment.

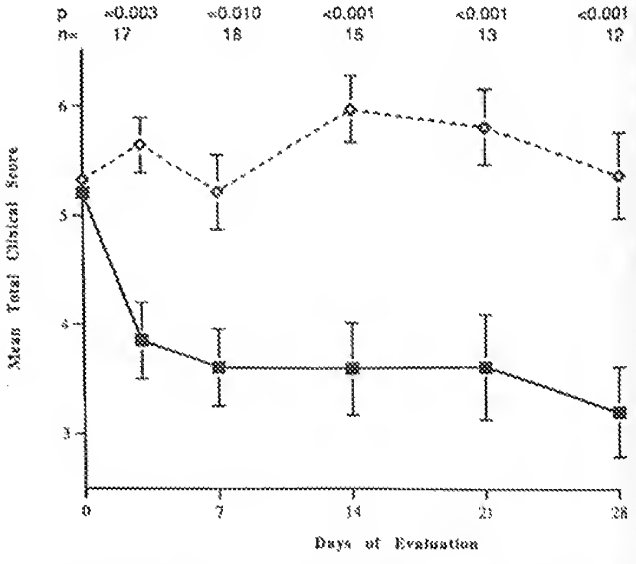

Figure 4. Time-course of change in clinical scores for active *versus* placebo-treated atopic dermatitis lesions. Longitudinal pattern of clinical response reflected by mean change (± SEM) from baseline total clinical scores of topical CP80,633 (■) *versus* placebo-treated atopic dermatitis (○) sites. Significant improvement was evident at each time point (p values indicated); n = number of subjects evaluated at each time point during therapy (days 3–28).

discontinued because of generalized worsening of the untreated dermatitis.

DISCUSSION

Atopic dermatitis, a chronic inflammatory skin disease, causes severe pruritus leading to excoriation and secondary infection. Economically, AD creates a considerable health care burden (Lapidus *et al*, 1993), accounting for 1% of pediatric outpatient visits (Sampson, 1990) and, because of lifelong cutaneous hyperirritability, over 80% of occupational skin disease (Shmunes and Keil, 1983; Nassif *et al*, 1994). Therapeutic options for AD, as for allergic respiratory disease, are limited and inadequate. Glucocorticosteroids are used almost exclusively, but toxic effects are evident in many patients. For some very severe cases, photochemotherapy, cyclosporin A, or IFN-γ are used, but these are high-risk, expensive, and generally unsatisfactory modalities. Safe, effective anti-inflammatory agents for treatment of AD are perhaps the greatest need and challenge in dermatologic disease.

Altered cyclic nucleotide metabolism in atopic disease was predicted previously by Szentivanyi (1968). This led to a series of studies that demonstrated blunting of cAMP responses in leukocytes of patients with AD. We showed that this defect was caused by high cAMP hydrolysis by PDE in atopic leukocytes (Grewe *et al*, 1982; Butler *et al*, 1983). The increased PDE activity was present even in cord blood cells of newborns from atopic parents (Heskel *et al*, 1984), indicating an intrinsic, possibly genetically controlled abnormality. We demonstrated that the increased PDE correlated with histamine hyper-releasibility and with elevated spontaneous IgE production in cultured AD leukocytes, and we showed that PDE inhibitors could normalize those functions (Butler *et al*, 1983; Cooper *et al*, 1985).

Other studies demonstrated that the atopic isoenzymes were distinctly more sensitive to each PDE-i (Giustina *et al*, 1984; Chau and Hanifin, 1993), suggesting that these agents have a therapeutic advantage in AD. Our studies have focused particularly on blood monocytes which have a major proportion of abnormal PDE activity. We recently presented evidence that AD monocytes also have a considerable immune modulating effect on T cells. IFN-γ production, which is reduced in MNL cultures, became normal or elevated in purified T-cell cultures, indicating a monocyte inhibitory effect on Type 1 T helper cells (Th1) (Chan *et al*, 1993). This led to the demonstration of increased spontaneous production of PGE_2 (Chan *et al*, 1993) and IL-10 (Ohmen *et al*, 1995), both known suppressors of IFN-γ production by Th1 cells.

These studies strongly suggest that increased PDE activity reduces intracellular cAMP levels that, in turn, allow greater basal monocyte secretion of T-cell modulators. Because of the association of increased PDE activity with the elevated PGE_2 and IL-10 production in AD monocytes, we reasoned that each PDE-i might correct these abnormalities. Comparisons in enzyme inhibition assays (**Table I**) showed that a new agent, CP80,633, was 10-fold more potent than the standard Type 4 inhibitor, Ro 20-1724. We found that CP80,633 had a greater inhibitory effect on PGE_2 and IL-10 production by AD monocytes and on IL-4 production in cultures of MNL. We cannot clearly state whether the IL-4 effect was indirect, by inhibiting monocyte modulating factors, or occurred directly on Type 2 T helper (Th2) cells. It could also be a combined effect, because we have observed PDE inhibition in lymphocytes, though this action occurred with a specific Type III inhibitor, olitraquazone (Chan and Hanifin, 1993), and CP80,633 is not an inhibitor of Type III PDE (Cohan *et al*, 1995).

It seems reasonable to consider that abnormal PDE isoenzymes underlie many of the immune and inflammatory abnormalities of atopic disease. We have demonstrated that inhibition of PDE influences a number of cellular and mediator pathways including monocyte PGE_2 and IL-10 synthesis, and IL-4 over-production. Because of CP80,633's effects in enzyme and cytokine inhibition and other predictive assays (Cohan *et al*, 1995), as well as the distinctly higher sensitivity of AD enzyme to PDE inhibitors, we tested the drug in clinical studies, comparing active 0.5% CP80,633 with placebo ointment vehicle applied to symmetrical right and left lesions in 20 patients with AD. Responses were prompt, showing statistically significant improvement within 3 d and maintaining throughout the 28-d trial. The drug reduced inflammation in 80% (16 of 20) treated sites, as compared to only 3 of 20 placebo sites. Importantly, no irritation or other adverse events were observed in a disease notoriously subject to irritancy.

Topical treatment is the preferred method for most patients with AD and is an area of great need because of the common involvement of face and eyelids, thin-skinned areas in which corticosteroids may cause atrophy. Systemic use of PDE inhibitors has been limited by side effects, particularly the common nausea and vomiting resulting from use of high-dose theophylline, and a particular problem with newer, more potent agents (Torphy and Undem, 1991); however, this high potency compound, CP80,633, clearly reduces atopic inflammation when applied topically on the skin. To date, we have noted no evidence of emesis or other side effects with topical use, though considerable absorption might be expected if large areas of skin were treated. Our study suggests that drugs of this type may also have potential for treating asthma via the inhalant topical route.

The clinical anti-inflammatory effectiveness by a potent Type 4 PDE-i applied to the skin confirms predictions from *in vitro* studies. This class of drugs, by increasing intracellular cAMP levels and reducing cytokine and mediator release, modulates exaggerated atopic responses by multiple immune and inflammatory cells. Single pathway inhibitors may be inadequate for controlling the many facets of inflammatory responses. It is hoped that this *in vivo* demonstration of efficacy will encourage development of useful alternatives to replace over-reliance on toxic corticosteroids in atopic disease.

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